only small pressures or vacua are developed is shown in Plate 1.

Pieces of tubing, mouths of flasks, etc., are simply flared and given a flat lip, and then this lip is grounded in the usual manner by emery on a flat glass surface. The customary small glass arms for anchorage of rubber bands for holding pieces together can be easily fused to the main element.

The writer has built "T's," "L's," and straight lengths of tubing of varying lengths which are all surprisingly interchangeable. Figs. 1 and 2 give a simple method for reducing the diameter of a tube—*i.e.*, by the insertion of a ground glass disk with a hole in it. The ground glass disk idea is also satisfactory where it is desired to have two tubes connect with one vessel or another tube. Fig. 3 shows a straight connection involving the same size tubing.

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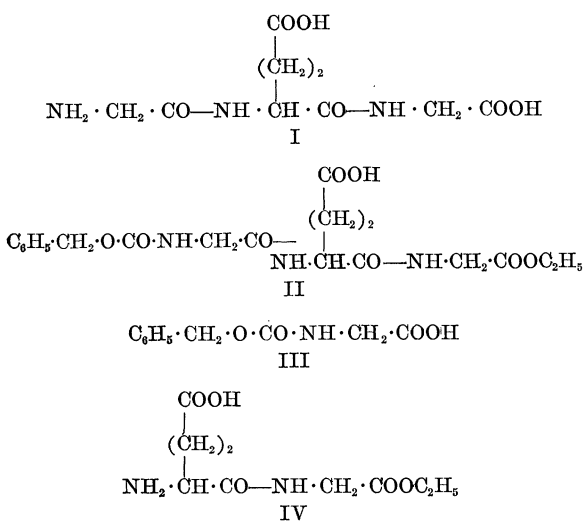
SPECIAL ARTICLES

A SYNTHETIC PEPTIDE AS SUBSTRATE FOR TRYPTIC PROTEINASE

LITTLE is known regarding the mechanism and the specificity of those enzymes which split true proteins —the proteinases. This is due to the fact that until recently it was not possible to obtain a proteinase substrate of known structure.

By means of the carbobenzoxy method[1] a peptide-like substrate for tryptic proteinase was synthesized. It is a derivative of the tripeptide glycyl glutamyl glycine (I). The amino group at one end of the molecule was blocked with the carbobenzoxy group, and the glycine carboxyl on the other end was esterified with ethyl alcohol (II). Thus the only free reactive group was the γ-carboxyl of the glutamic acid.



Substance II was split quite rapidly by pancreatin Merck as well as by a preparation of crystalline trypsin (tryptic proteinase) kindly placed at our disposal by Dr. John H. Northrop. The products of the splitting were carbobenzoxy glycine (III) and glutamyl glycine ester (IV). The latter product is rapidly transformed to a diketopiperazine under the conditions of the experiment.

[1] M. Bergmann, SCIENCE, 79: 439, 1934.

This experiment shows that tryptic proteinase does not require for its action linkages of unknown nature, but is able to split ordinary peptide linkages if the rest of the molecule fulfils certain structural requirements. In II one of these requirements is the presence of the free γ-carboxyl, which combines with the tryptic proteinase and thus enables it to split the peptide.

It is probable that the other amino dicarboxylic and diamino carboxylic constituents of the proteins play a rôle similar to that of glutamic acid in combining with proteinases by means of their extra acid or basic groups. From the work of Gurin and Clarke[2] it is to be expected that the ε-amino group of lysine in a protein combines with pepsin. By means of the carbobenzoxy method we are preparing peptides of lysine and aspartic acid and shall report on their behavior towards proteinases in the near future. The theoretical significance of these results as well as the interesting experiments of Matsui,[3] Ishiyama[4] and Shibata[5] on the splitting of diketopiperazines will be discussed in a future publication.

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THE ELECTRICAL RESPONSE OF THE VESTIBULAR NERVE DURING ADEQUATE STIMULATION

A STRIKING characteristic of the vestibular nystagmus which is produced in virtually all vertebrate species by the angular retardation incident to the termination of a prolonged period of uniform bodily rotation (also by the acceleration incident to the onset of such a period of rotation) is that this response ordinarily persists for a considerable time—often 20 or 30 seconds—after the cessation of its

[2] S. Gurin and H. T. Clarke, *Jour. Biol. Chem.*, 107: 395, 1934.
[3] J. Matsui, *Jour. Biochem.*, 17: 163, 253, 1933.
[4] T. Ishiyama, *Jour. Biochem.*, 17: 285, 1933.
[5] K. Shibata, *Acta Phytochimica*, 8: 173, 1934.

objective stimulus. In order to account for the discrepancy between the duration of this response and the duration of its stimulus, two general theories have been advanced. The *peripheral* theory holds that the post-stimulus persistence of vestibular nystagmus is dependent upon a continuation of the excitatory process (inertial movements of the endolymph, displacement of the cupula, or the like) which is initiated in the vestibular receptors by the objective stimulus. The *central* theory, on the other hand, holds that the post-stimulus persistence of this response is dependent upon the action of a neural mechanism located within the brain, which, once properly excited by a stimulus, however brief, continues to transmit effective impulses to the muscles involved in nystagmus until either this "after-discharge" mechanism becomes self-damped (perhaps through a gradual lengthening of the refractory phase of the constituent neurons) or until its activity is checked by the occurrence of an opposing stimulus.

In the hope of obtaining possibly more definitive evidence than has previously been advanced in support of either of these two rival hypotheses, the writer has undertaken a systematic comparison of the duration of objective stimulation of the vestibular receptors and the duration of the action currents thereby produced in the vestibular nerve. By means of a vacuum-tube amplifying apparatus developed by Dr. E. G. Wever and Dr. C. W. Bray, of Princeton University, for use in their investigation of the electrical phenomena of the auditory nerve, it has been found possible to make the passage of impulses along the vestibular nerve audible in a telephone receiver. The results thus far obtained from the study of the common painted terrapin (*Chrysemys picta*) indicate that, at least in this type of subject (selected because of particular accessibility of the vestibular nerve), the action currents probably never last for more than a fraction of a second after the cessation of objective stimulation. When the terrapin is accelerated on a manually operated turntable, there is audible in the receiver a distinct burst of discharge; and when the animal is retarded there is audible another similar burst of discharges, even though the maximum angular velocity attained be quite moderate. During prolonged rotation weaker discharges may be heard more or less continuously, due presumably to the more or less constant stimulation of the vestibular receptors through slight unavoidable variations in the speed of rotation. However, at the end of rotation, objective stimulation definitely ceases; and in no case has the passage of impulses been heard for more than approximately half a second after the subject has come to rest.

It is conceivable, of course, that effective impulses may continue to pass over the vestibular nerve (due to a continuation of the excitatory receptor process) for many seconds after the end of objective stimulation, without necessarily being detectable by the technique just described. However, if further investigation, with oscillographic recording and a more accurately controllable method of stimulation, confirms the results thus far obtained, it will be reasonably certain that the post-stimulus persistence of vestibular nystagmus is due, not to a concomitant persistence of receptor activity, but rather to sustained after-discharges from a neural mechanism located within the brain.

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THE SPECIAL REACTIVITY OF PEPTIDES

IT is one purpose of the present note to point out that, whereas current theory seems to assume that most of the obvious transformations of amino-acids occur when these are present as such, some of these reactions may really take place much more readily as transformations of peptides.

For some time the writer has been interpreting the decomposition[1] of cysteine and cystine derivatives by alkalies as a reaction in which preliminary enolization allowed expulsion of the sulfur (with whatever might be attached to it) as a negative group. Theory and recorded fact seemed to agree as to the modifications of the amino- and carboxy- groups necessary to produce greatly increased reactivity. Peptides (and analogous compounds) were conspicuously more reactive than simple amino-acids, though less reactive (for reasons which will be explained elsewhere) than cyclic derivatives, such as diketopiperazines and hydantoins.

From the beginning it was assumed[1,2] that this type of reaction would be reversible. It has now been found possible[3] to add p-tolylmercaptan to α-acetylaminoacrylic acid to form S-p-tolyl-N-acetylcysteine, and this is now leading to a new cystine synthesis which will perhaps have a definite relation to the natural synthesis of cystine. The important point in the present connection is, however, that it is decidedly easier to add[3] mercaptans to benzoyl-dehydrophenylalanyl-glycine ester, which may be considered as a model of a dehydro-tripeptide. The addition as well as the elimination of sulfur derivatives thus occurs according to the principles already advanced. That is, it occurs more readily in a peptide which is at least a tripeptide, and in which the active portion of the molecule is not in a terminal position.

[1] B. H. Nicolet, *Jour. Am. Chem. Soc.*, 53: 3066, 1931.
[2] B. H. Nicolet, *Jour. Biol. Chem.*, 95: 389, 1932.
[3] Unpublished results.